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FUNCTIONS OF THE PROTEIN AND OTHER NITROGENOUS FRACTIONS OF POTATOES IN CHIP COLOR DEVELOPMENT[1]

It is generally accepted that the color of potato chips is a result of the "browning" or Maillard reaction which occurs largely between amino acids and reducing sugars. Ramsey *et al* (4) indicated that casein and glucose may react in a similar manner to that of amino acids and sugars. Mohammed *et al* (3) also reported that the browning reaction with proteins occurred in a system of bovine serum albumen-glucose solution.

The present studies were conducted with the following objectives: 1) to prepare potato protein fraction in sufficiently pure form for browning reaction experiments, 2) to determine the means by which the potato protein fraction participates in browning reactions during chip frying and the relative reactivity of the protein nitrogen and the non-protein nitrogen and 3) to determine the extent of the browning reaction of the potato protein fraction with glucose, fructose and sucrose using a model system to simulate conditions prevailing in chip frying.

METHODS AND RESULTS

Preparation of Potato Protein Fractions: At each date of sampling six treatments were involved, namely, three harvest dates, September 26, October 14 and November 9, and two storage temperatures, 40° and 50° F. The six treatments were replicated twice. Potatoes were sliced and frozen immediately in crushed dry ice. Samples were freeze dried and stored at 0°.F. until used.

A weighed portion of the dry potato powder was mixed with four times its weight of water plus 0.5 per cent sodium bisulfite and allowed to stand at 32° F. overnight with occasional shaking. The sample was then filtered through a Buchner funnel using filter aid and the amount of filtrate measured. It was assumed that soluble protein was equally distributed in the filtrate and in the water remaining in the filter pad. Therefore, instead of washing the filter pad or re-extracting in an attempt to get 100 per cent recovery (which was found to be impractical), in subsequent calculations a correction was made for the amount of water left in the funnel.

Sodium sulfate was added to the filtrate to precipitate proteins. It was found that Na_2SO_4, as used by Howe (1) in the old method of isolating plasma proteins, tended to give a precipitate that was easier to centrifuge than did ammonium sulfate. It was further found that, in the absence of a refrigerated centrifuge, it was better to do this step at room temperature to avoid changes in solubility of the precipitate as the solution increased in temperature.

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[2]Cornell University, Ithaca, N Y., and Eastern Utilization Research and Development Division, Agricultural Research Service, U.S.D.A., Philadelphia 18, Pa., respectively.

The sodium sulfate precipitate was suspended in water and dialyzed for three days at 32° F. During this time the suspension first cleared, and then a precipitate was formed. After three days, the contents of the dialysis was centrifuged. The supernatant portion was decanted and made up to volume. This was the water soluble protein and corresponds to Levitt's albumen fraction. The precipitate was washed three times with water and dissolved in 1 M NaCl. This was designated NaCl-soluble or water-insoluble protein and corresponds to Levitt's (2) globulin fraction.

The water soluble protein was colorless or slightly milky. The precipitated NaCl-soluble protein was white, appearing slightly turbid in salt solution, and tending to darken if allowed to stand several days at room temperature, indicating the possible presence of tyrosinase.

The pH of the solutions during extraction and dialysis, as well as the pH of the two protein fractions, was about 5.8. To determine whether there was protein in the water soluble fraction, a trichloroacetic acid precipitation was made. It was found that one-third of the nitrogen in this fraction was precipitable protein while the remainder was non-precipitable protein or peptides

The protein solutions were analyzed for nitrogen by the micro Kjeldahl method, and the results presented in Table 1.

Relative Reactivity in the Browning Reaction of Potato Protein Nitrogen and Non-protein Nitrogen Alone and with Glucose, Fructose and Sucrose.

The protein solutions were used within three days after dialysis was completed. Whatman No. 1 filter paper discs were dipped into the solutions and subsequently fried for two minutes at 380° F. The protein solutions were used alone and in combination with 0.05 M glycine and 0.05 M glucose, fructose and sucrose. The discs were washed with aliquots of carbon tetrachloride until a constant reading was obtained on the Hunter Color Difference Meter. Data of the January 16 sampling are presented in Tables 2 and 3. The data of the harvests of October 14 with potatoes stored at 40° F., November 9 with potatoes stored at 50° F. and October 14 with potatoes stored at 40° F. until April 23 followed by three weeks storage at 75° F. are presented in Table 4.

The original protein extraction of the first sampling on January 16 was intended to be a split, split plot analyzed by covariance, using color of discs from solutions without protein as the independent variable. Midway through the extractions it was apparent that protein was adding nothing, at least visually, to the color of the filter paper discs, therefore, only one replication was made. Consequently, time of harvest and storage temperature were confounded with extraction procedure, but since the difference was not significant statistically for soluble protein ($F = 2.15$ at 2 and 2 d.f. for date and $f = 1$ at 1 and 2 d.f. for temperature) this does not matter. Also since replications were dropped, the error term used to test date x temperature is deceptively small. However, the interaction is not significant ($F = 2.5$ at 2 and 83 d.f.) for soluble protein and would be still smaller if the error was correct.

The split plots, which are the different solutions, present a problem in analysis. Values were transformed to log $(1/Rd \times 10^2)$. There is a significant difference between discs dipped in solutions containing soluble protein and in those without soluble protein. It seems most likely, however, that

TABLE 1.—*Protein fractions of potatoes of three harvests and two storage temperatures.*

Harvest date	Storage temperature	Protein (6.25 x N) as per cent dry weight	
		H_2O soluble	NaCl soluble
Sept. 26	50°	5.21	3.52
Oct. 14	50°	--	--
Nov. 9	50°	4.77	--
Sept. 26	40°	5.16	3.50
Oct. 14	40°	4.33	3.70
Nov. 9	40°	5.24	--

TABLE 2.—*Color of filter paper discs (Rd)*fried after dipping in soluble protein fraction of potato tubers from three harvests and two storage temperatures, with and without glycine, glucose, fructose and sucrose (Jan. 16 sampling).*

Disc Number and Treatment	Stored at 50° F.			Stored at 40° F.		
	Date of Harvest			Date of Harvest		
	Sept. 26	Oct. 14	Nov. 9	Sept. 26	Oct. 14	Nov. 9
1. Water	61.7	61.3	60.8	62.3	61.9	60.8
2. Protein	62.0	60.4	61.4	62.1	60.6	61.8
3. Protein-glucose	59.5	59.7	59.2	60.0	59.2	58.8
4. Protein-fructose	60.5	55.6	59.8	58.7	57.6	58.8
5. Protein-sucrose	62.1	58.6	60.6	61.5	61.0	60.3
6. Protein-glycine	60.5	57.9	58.9	60.2	58.7	59.2
7. Protein-glucose-glycine	20.4	20.1	27.1	23.2	20.6	23.5
8. Protein-fructose-glycine	20.0	20.4	24.2	21.4	20.3	20.2
9. Protein-sucrose-glycine	50.4	34.5	43.4	41.0	34.7	35.9
10. Glucose	62.0	61.3	61.1	61.7	61.2	60.5
11. Fructose	62.5	61.0	61.8	61.8	61.3	60.2
12. Sucrose	63.4	61.1	61.6	62.1	60.9	61.6
13. Glycine	61.8	57.0	60.3	59.4	58.2	57.9
14. Glucose-glycine	21.7	20.2	22.9	23.2	22.0	20.3
15. Fructose-glycine	19.6	22.2	23.9	22.8	21.2	22.1
16. Sucrose-glycine	50.2	36.5	51.5	49.9	41.2	41.5

*White discs have Rd of 60 or above. The lower the Rd, the darker the disc.

the increase in color is due to the fact that the solutions remained in the cold room several days before the fryings were made. There are two points that bear this out, first, the fact that there is no difference between the various protein solutions although we know from the chemical analysis that the protein solutions differed in amounts of nitrogen and, second, the results from the second protein sampling.

Only the October 14 harvested tubers stored at 40° and the November 9 harvested tubers stored at 50° F. were extracted for protein the second time, on April 23. The protein solutions were fried on filter paper discs within 24 hours after dialysis. When paired samples were analyzed separately by the "t" test there was no significant difference (t value of 0.4 to 0.6 for 7 d.f.). Since protein contributed nothing to the color of the discs in this experiment, it seems doubtful that it had any effect in the others.

TABLE 3.—*Color of filter paper discs (Rd)* fried after dipping in insoluble protein fraction of potato tubers from three harvests and two storage temperatures, with and without glycine, glucose, fructose and sucrose. (Jan. 16 sampling).*

Disc Number and Treatment	Stored at 50° F.			Stored at 40° F.		
	Date of Harvest			Date of Harvest		
	Sept. 26	Oct. 14	Nov. 9	Sept. 26	Oct. 14	Nov. 9
1. Water	62.4	60.5	60.7	63.7	63.4	59.8
2. Protein	61.6	59.8	61.7	62.6	63.3	60.7
3. Protein-glucose	61.3	59.6	60.0	62.4	61.7	60.6
4. Protein-fructose	57.8	59.6	58.9	60.6	63.1	59.9
5. Protein-sucrose	60.8	59.6	60.5	62.4	63.4	60.5
6. Protein-glycine	57.3	57.2	57.2	58.4	58.4	58.5
7. Protein-glucose-glycine	20.9	28.6	24.7	24.6	22.3	26.0
8. Protein-fructose-glycine	28.6	23.1	24.3	23.6	23.1	26.7
9. Protein-sucrose-glycine	27.8	42.2	34.6	38.6	37.1	43.6
10. Glucose	61.9	59.7	61.3	61.4	62.6	60.3
11. Fructose	60.4	59.5	60.8	63.1	61.9	61.1
12. Sucrose	61.8	61.0	60.6	62.4	63.1	60.7
13. Glycine	57.0	56.3	57.7	58.8	57.9	58.0
14. Glucose-glycine	23.0	24.3	24.6	26.8	26.5	25.8
15. Fructose-glycine	24.0	29.5	23.5	18.5	20.8	25.9
16. Sucrose-glycine	41.8	39.1	32.9	38.0	36.3	41.6

*White discs have Rd of 60 or above. The lower the Rd, the darker the disc.

Insoluble and soluble protein values were analyzed statistically, "t" being about the same in both cases.

Tubers harvested October 14 and stored at 40° F. were reconditioned at 75° F. for three weeks at which time they made chips with an Rd reading of 24.8, compared to an Rd of 3.2 before reconditioning. Proteins were extracted in the usual manner and fried in combination with sugars and glycine on filter paper discs.

The water soluble protein sample was combined with 0.01 M sucrose and 0.23 M glycine instead of the usual 0.05 levels of each. Glucose and fructose concentrations remained at 0.05 M, however. The sodium chloride soluble protein was combined with sugars and glycine (0.05 M) separately, but not with both glycine and sugars at the same time.

For some reason, all the solutions, including the checks, fried considerably lighter in color than previously. Table 4 presents the data for color (Rd) of filter paper discs fried with the various combinations of sugars, glycine and protein fractions.

Using Student's "t" to compare paired samples with and without protein showed that neither soluble nor insoluble protein was significant. ($t = 0.5$ and 0.1 at 7 and 5 d.f.).

By visual observation, there appears to be no effect of the protein on browning in the model system. If discs from solutions 1 and 2, 6 and 13, and 7 and 14 (Tables 2 and 3) are compared, little difference can be detected. There certainly appears to be no difference between temperatures and harvest date treatments as a result of the protein fractions.

Rd readings of the Hunter Color Difference Meter show also that

TABLE 4.—*Color of filter paper discs (Rd)*fried after dipping in soluble protein and insoluble protein fractions of potato tubers from two harvests and two storage temperatures, with and without glycine, glucose, fructose and sucrose (April 23 sampling).*

Disc Number and Treatment	Harvested Oct. 14 Stored at 40° F.		Harvested Nov. 9 Stored at 50° F.		Harvested Oct. 14 Stored at 40° until April 23, 3 weeks at 75° F.	
	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
1. Water	61.2	65.5	61.5	64.3	81.0	80.3
2. Protein	60.1	64.0	60.8	65.5	81.4	84.4
3. Protein-glucose	61.2	65.8	60.3	63.4	79.9	84.6
4. Protein-fructose	61.1	65.8	63.2	63.3	81.5	81.6
5. Protein-sucrose	61.2	65.0	59.3	63.8	81.2	83.1
6. Protein-glycine	58.4	62.3	59.3	61.9	75.4	80.6
7. Protein-glucose-glycine	31.0	31.7	30.3	28.5	32.8	..
8. Protein-fructose-glycine	23.5	29.9	27.5	28.3	33.1	..
9. Protein-sucrose-glycine	51.0	56.2	53.8	49.5	72.0	..
10. Glucose	61.2	66.2	60.1	61.8	80.7	84.0
11. Fructose	61.3	64.0	59.4	64.7	79.6	84.3
12. Sucrose	61.8	66.8	61.2	63.2	83.2	84.4
13. Glycine	59.6	63.0	59.1	62.8	78.2	82.3
14. Glucose-glycine	27.6	28.3	28.4	31.9	31.4	..
15. Fructose-glycine	27.6	33.5	22.6	37.4	33.1	..
16. Sucrose-glycine	56.0	55.3	54.9	54.1	78.2	..

*White discs have Rd of 60 or above. The lower the Rd, the darker the disc.

neither the soluble nor the insoluble protein fraction participates to any degree in the browning reaction. Numerous comparisons such as the following indicate this: 1 *vs* 2; 2 *vs* 10; 4 *vs* 11; 5 *vs* 12; 6 *vs* 13; 7 *vs* 14; 8 *vs* 15; 9 *vs* 16. This relationship holds regardless of date of harvest or storage temperature.

The only treatments that result in extensive browning reaction are those containing glycine with either glucose or fructose and to a lesser extent, with sucrose.

The earlier preliminary results in which there was some darkening can probably be explained as a result of growth of microorganisms. These solutions had been allowed to stand for quite some time before frying.

SUMMARY AND CONCLUSIONS

Both by visual observation and by Hunter Color Difference Meter measurements (Rd) there appears to be no effect of the protein, soluble or insoluble fractions, on browning in the model system. There also appears to be no difference in color of filter paper discs between storage temperature and harvest date treatments as a result of the protein fractions.

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